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BIORT: An experiment for the assessment of the biological effects of very high dose rate and dose per pulse electron irradiations

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Summary. — Intra Operative Radiation Therapy (IORT) is a treatment modality combining surgery and radiation therapy in which a large single dose of radiation is delivered at the time of an operation for tumour resection. During last years, IORT has become a widespread technique in clinical routine. This was possible thanks to the recent development of small dedicated electron accelerators which overcome all the disadvantages concerning both organizational and radiation protection issues related to the use of conventional Linacs during a surgical operation. However the very high dose per pulse and dose rates delivered by the this new modality (*i.e.* respectively about 100 and 20 times greater than that of a conventional radiation therapy) open new problems related to dosimetry and radiobiology. For these very reasons we proposed the experiment "radioBiology of IORT" (BIORT) to INFN, aiming at determining biological effectiveness of the very peculiar IORT beams (electrons delivered at very high dose rate, high dose per pulse and a total dose delivered in a single fraction).

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1. – Introduction

Intra Operative Radiation Therapy (IORT) is a cancer treatment modality referring to radiotherapy in which a large dose of radiation is delivered to the target volume in a single fraction at the time of surgical resection of a tumour. The tumour bed can then be sterilised or unresectable tumours can be directly irradiated. The advantages of such a technique with respect to the conventional radiotherapy (irradiation with external

beams and with fractionated dose) can be summarized in three principal aspects: increase of the local tumour control; decrease of toxicity to normal tissues; to avoid the long radiotherapy treatment after operation, when the case.

Its use dates back to the beginning of last century and its modern approach started in the 1960s. Nevertheless its success and then use was quite limited principally because of the logistic and anaesthesiology difficulties connected to the necessity to move patients from the operation theatre to the radiotherapy bunker.

More recently a renewed interest has grown and still is, following the development of dedicated small linear accelerators which overcome the above-mentioned problems as dedicated Linacs can be placed in the operation room and even be moved to one room to another.

Furthermore, IORT is in fact a very peculiar radiotherapy technique implying the delivering of very high dose to the target volume in a single fraction during the surgical excision of the tumour and also dose rate and dose per pulse are considerably higher than in conventional radiotherapy since the treatment should last for a few minutes. This clearly poses radiobiological and dosimetric questions that have to be faced.

The present main limit of the IORT treatment resides in the impossibility to prepare in advance a specific treatment planning for dose distribution evaluation. Since the target volume is determined directly in the operation room just before irradiation, at that very time the electron beam energy, applicator and total dose are chosen to ensure the best target dose distribution.

Currently, in Italy more then twenty centres with dedicated accelerators to IORT are operative.

2. – IORT rationale and technique

IORT rationale and technique have been discussed in detail in some recent review papers [1-4]. Briefly, the advantages of such a technique with respect to the conventional radiotherapy (irradiation with external beams and with fractionated dose) can be summarized as follows:

- improved local control of the illness;
- reduced absorbed dose delivered to normal tissues;
- improved therapeutic ratio, that is the index for tumour response for a fixed level of normal tissue damage.

This is made possible by a direct surgical definition of the treatment target and the possibility of displacing or shielding healthy tissues allowing a very well conformed irradiation, and avoiding, at the same time, the exposure of healthy contiguous tissues. As a result, an enhanced tumour control is expected.

IORT is usually performed together to an External Beam RadioTherapy (EBRT), but at present an interest is emerging for its application as the sole radiation therapy in conservative treatment of early-stage breast cancer.

In particular IORT is appropriate when surgery is not expected to give a reasonable local control or when very high doses in external beam therapy, which can eventually be unacceptable for the normal tissue, are necessary. Furthermore in case of early stage cancer breast, IORT seems to be a valid alternative to the conventional external beam irradiation after surgery.

Three types of IORT dedicated Linacs generating high-energy pulsed electron beams became recently available for clinical use.

The first device developed as IORT dedicated accelerator is the Mobetron (IntraOp, USA), whose characteristics are indeed very similar to a conventional Linac. It can provide four energy beams up to 12 MeV with 0.4–0.6 cGy/pulse, and 10 GHz frequency. However its huge weight (~ 1400 kg) and its not negligible scattered radiation considerably limits its use.

The second type of accelerator is the Novac7 (Hytesis, Italy). This machine can generate electron pulsed beams in the nominal energy range from 3 to 9 MeV with a frequency in the 1–30 Hz range (5 Hz is the frequency in the clinical routine) and 4 μs pulse width.

The Novac7 head is devoid of magnetic lenses and scattering foils and the beam collimation is obtained using special cylindrically shaped perspex collimators (applicators) of different sizes.

The dose per pulse can reach 13 cGy/pulse (for comparison, the dose per pulse in conventional Linacs is 0.1 cGy/pulse) and the dose rate can reach 40 Gy/min using the IORT clinical routine pulses frequency (against the 2 Gy/min of the conventional Linacs). Its weight is 650 kg, so operation rooms do not need to be reinforced.

The third dedicated accelerator is the Liac (Info & Tec, Italy). Its characteristics are similar to those of Novac7 and it is even lighter (~ 400 kg). We can mention three main differences from Novac7. The first one is the nominal maximum energy value of 10 MeV or 12 MeV, according to the version, allowing the irradiation of about 3 cm or 4 cm of water equivalent tissue with 90% isodose. This characteristic is very useful for treatment of relatively thick targets. Furthermore a 85 μm thick brass diffusive filter is present for a better entrance dose distribution uniformity. Finally Liac has a usual working frequency of 10 Hz, thus reaching a dose rate up to 20 Gy/min.

The technical characteristics of both Novac7 and Liac show useful practical advantages: they can be easily moved, they do not present particular problems from the radiation-protection point of view, the very high dose rate allows treatments within a few tens of seconds, a very important feature as the treatment has to be done on anesthetized patients. The high entrance percentage dose value allows a good coverage of the target and photon contamination is very low ($< 1\%$).

Treatments are performed with a different set of applicators in accordance with the superficial dimension of the tumour.

3. – Experiment proposal

The very high dose per pulse of a dedicated IORT Linac, and the delivery of very high dose in a short single fraction pose radiobiological questions, in particular on the biological effectiveness of IORT beams.

In fact, the biological equivalent dose for treatment is determined on the basis of the linear-quadratic model of cell clonogenic survival curve, that is the model generally used to predict radiation effects, based on the α/β ratio, the parameters of the dose-effect curve. Accordingly, the dose D_{IORT} to be delivered in one fraction IORT treatment is related to the total dose D_{EBRT} in a conventional fractionated EBRT delivering 2 Gy per fraction by the formula [3]

$$D_{\mathrm{IORT}} = (1/2)\left\{\left[(\alpha/\beta)^2 + 4D_{\mathrm{EBRT}}\,(\alpha/\beta + d_f)\right]^{1/2} - \alpha/\beta\right\}, \tag{1}$$

where $d_f = 2$ Gy.

Anyway possible biological effects due to the delivery modality of IORT, that is due to the IORT beam quality are not taken into account.

As far as dosimetry is concerned, according to the international recommendations, the dosimetry of beams by dedicated IORT accelerator cannot be performed with ionization chambers. The standard method used for charge loss evaluation in an ionization chamber is not feasible in the actual high dose per pulse and high gradient dose typical in IORT [5-8].

Moreover, in such physical conditions the ionization chamber correction factors for absorbed dose measurements are not available. Factors used for conventional electron beams are, in principle, not appropriate being the characteristics of IORT and EBRT very different.

Current IORT guidelines suggest the use of dose-per-pulse independent dosimeters, such as the chemical Fricke dosimeter. However due to its low sensitivity, it requires long-lasting irradiation eventually causing radioprotection problems.

However the possibility of the use of the parallel plate ionization chambers for the absolute and relative dosimetry of Novac7 electron beams could have a clear practical importance, particularly considering the typical instability of the IORT beams in terms of electron number per pulse, which is about 1–2% within a day, 4–5% within a week, and about 8% in a year. The use of the ionization chamber could allow the on-line dosimetric information, which is essential for the long-lasting radiobiological irradiations proposed in our experiment. Recently a new method for the accurate evaluation of the correction factor (k_{sat}) taking into account the loss of collected charge due to the ion recombination has been already shown by some of the BIORT proponents [9]. They derived a general equation for k_{sat}:

$$k_{\mathrm{sat}} = \frac{\ln(p(e^{\alpha q_\theta} - 1) + 1)}{\alpha p q_\theta}\,. \tag{2}$$

This new equation depends only on known or measurable quantities: α is tabulated, being only related to the characteristic of the ionization chamber and its supply voltage, q_θ is the collected charge per pulse, and p is the free-electron fraction, electrons that escape attachment to gas molecules reaching the electrode as free electrons. p depends on the chamber characteristics and a method for its evaluation for a given chamber is also shown in the same paper.

Moreover it has already been shown [10] the possibility to realize absolute dose measurements by means of a small cylindrical ionization chamber, independently of the beam direction.

3˙1. *Radiobiology of very high dose rate and very high dose per pulse: the state of the art and proposed measurements.* – In the past biological effects due to dose rate have been clearly assessed in the range 0.01–10 Gy/min in terms of repair of sub-lethal damage [11]. At higher dose rate some pioneering studies in the 1960s [12, 13] show some reduced biological effect due to the high dose rate which could be explained with oxygen consumption by radiation and the important free radical formation due to the high dose rate which give rise to a most probable recombination. Nias *et al.* showed instead no differences in radiosensitivity of HeLa cells after irradiation with 10 ns electron pulses [14].

Even more recent studies (see, for example, [15-18]) are not in a qualitative agreement, are difficult to compare, owing to their different irradiation conditions, and sometime

dosimetry is not very clear. Schulz *et al.* [15] studied clonogenic survival of Chinese-hamster lung cell after 250 kV X-rays and pulsed 30 MeV electron beam with a dose rate in the range $(0.5–5) \times 10^8$ Gy/s. They found the electron beam biologically less effective than X-rays, but they could not relate their results to oxygen consumption. At similar experimental condition (35 MeV electron beam and $(0.25–2.5) \times 10^8$ Gy/s) Purdie *et al.* [16] found an opposite result. They measured a significant decrease in human kidney cell survival as a function of dose rate both comparing γ-rays irradiation at different dose rate, and comparing the reference radiation to the pulsed electron beam. Early skin reaction in mouse has also been studied [17] with the result of an increase of the biological effect up to the dose rate of 0.96×10^8 Gy/s with a decrease at even higher values, probably due to oxygen effects. Finally Zackrisson *et al.* [18] did not find any significant difference in the RBE and OER in their study of clonogenic survival of V-79 cell line performed with 50 MeV electrons and 3.8×10^2 Gy/s mean dose rate and reference radiation.

In conclusion, a coherent picture does not exist about biological effects of very high dose per pulse and high dose rate. Furthermore, even effects due to a very high single dose should be addressed as emphasized in [19] where a possible combined effect of high dose rate and high single dose is envisaged. Moreover a recent paper [20] stressed the concern of the effects of single high doses both to tumour and normal tissue, being late effects to normal tissue an open question, in case it remains in the field of treatment.

On the basis of what is set out above we think that a systematic study on this field is urgently needed at IORT accelerator, and in particular we propose to radio-biologically characterise the IORT beam by the Novac7 accelerator, where dose delivery to the patients has a dose per pulse in the range 3–13 cGy/pulse.

We propose to measure cell survival on the MCF7 cell line, that is a breast cancer carcinoma cell line being early breast carcinoma one of the selected tumour to undergo IORT.

We plan to perform irradiation with conventional X-rays, electron for conventional therapy and finally with IORT electron in three different experimental set-ups: we will perform our cell irradiations with three different beams: X-rays with maximum energy of 250 keV; electrons produced by conventional Linac with $E_0 = 7$ MeV (E_0 is the mean energy at zero depth in water); electrons accelerated by a dedicated to IORT Linac with the same E_0. Our goal is to assess:

- Biological effects due to beam quality. Survival curves will be measured, in the dose range (0–10) Gy, with 250 kVp X-rays, conventional external electron beam accelerated by the Primus Linac and with electron from Novac7 at similar condition of conventional electrons: $E = 7$ MeV, dose rate 2 Gy/min, dose per pulse 0.1 cGy/pulse.
- Biological effects due to dose rate. Survival curves will be measured, in the dose range (0–10) Gy at Novac7, at three different dose rates: 0.4 Gy/s (typical value in IORT treatments); 0.8 Gy/s (maximum value in IORT treatment); 1.6 Gy/s (maximum reachable value).
- Biological effects due to dose per pulse. Survival curves will be measured, in the dose range (0–10) Gy at Novac7, at a fixed dose rate of 0.4 Gy/s and at two values of dose per pulse: 2 and 6 cGy/pulse.
- Biological effects due to single high dose as a function of dose rate. Survival of megacolonies [20] will be measured at Novac7 after a 20 Gy dose delivered at three different dose rates (0.4 Gy/s, 0.8 Gy/s, 1.6 Gy/s).

A study aiming at understanding these radiobiological issues is then crucial since in the clinical routine, dose per pulse and dose rate can vary according to the accelerator, the energy and the applicators; therefore an RBE dependence, for example, on the dose per pulse could lead to differences in dose equivalent among patients receiving the same treatment.

3˙2. *Dosimetry.* – In our experimental situation the dosimetry needed to quantify the absorbed dose by the irradiated culture cells will consist of dose measurements in reference conditions, that is measurements will be performed in a water phantom at a reference depth in water, and at standard conditions of temperature, pressure and humidity. Afterwards, measurements or calculations of the correction factors due to the difference between the reference and experimental conditions will be carried out.

In particular, for dosimetry at experimental conditions the perturbation of beam spectra and the fluences caused by cell culture flasks must be quantified together to the variation of the output of the accelerator with time and the environmental conditions.

The reference dosimetry of the 250 keV X-rays and the 7 MeV conventional electrons are well described in the AAPM International Report [5]. An accuracy of 2–3% in dose measurements can be reached for the 250 keV X-rays and of 3% for electrons (reference condition). As concerning the X-rays, the correction factors taking into account the experimental condition are negligible (the total accuracy of the absorbed dose will be about 3%) while they must be quantified for electrons. In this case the perturbation factors of the experimental conditions will be measured with EBT Gafchromic [21] dosimeters and we expect to reach an accuracy of about 4% in the evaluation of the cell absorbed dose.

On the other hand, dosimetry of electrons produced by the IORT Linac is critical for the following reasons: the very high dose per pulse does not allow the use of the International protocol for dosimetry [5].

Accelerator stability is very weak in comparison to conventional Linac; the energy spectrum is more degraded with respect to a conventional Linac beam with the same E_0 [22]. Indeed the entrance dose in water is greater for Liac (about 90%) and Novac7 (about 85%) with respect to that of electron beams produced by conventional Linac (around 80%). Consequently a greater beam perturbation is expected due to the presence of the cell flasks.

For these reasons, dosimetry in reference conditions and the assessment of possible output variation with time and environmental conditions will be performed as described in recent papers [9,10] with a final accuracy of about 3% for absorbed dose measurements. The correction factor evaluations taking into account the loss of collected charge in the ionisation chamber will be carried out both with Monte Carlo simulation, and direct dose measurements with Gafchromic EBT. We expect a total absorbed dose accuracy of about 5%.

4. – Conclusions

In this paper we have described the new experiment: "radiobiology of IORT" (BIORT) recently approved by the Istituto Nazionale di Fisica Nucleare (INFN). The goal of BIORT is the assessment of the biological effect of the IORT beams accelerated by dedicate electron accelerators by measurements of the clonogenic survival of the MCF-7 cell line. The importance of this study resides on the fact that IORT is now a quite widespread radiotherapeutic technique (thanks to the mentioned dedicated accelerators) against the absence of systematic radiobiological characterisation of these very peculiar beams (very high dose rate and very high dose per pulse).

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